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DESIGN, SYNTHESIS AND TESTING OF METABOLICALLY-STABLE ANTIMALARIAL COMPOUNDS

by

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13. ABSTRACT

The goal of this research is to contribute to the development of a new, more effective antimalarial drug. Malaria kills an increasing number of people each year, due in part to the ability of the parasite that causes it to develop resistance to the drugs used to treat the disease. It is therefore essential that new treatments be developed which employ novel compounds. This research is based upon one class of compounds called chalcones, which are known to kill the malaria parasite by a mechanism different from that used by existing antimalarial drugs. This means that the parasite will not have any resistance to a chalcone-based drug when it is first deployed. In this research, a series of six novel organic compounds were designed that would possess both antimalarial properties and stability in the presence of metabolizing enzymes: both requirements for a useful drug. Beginning with commercially available compounds, a four-step laboratory synthesis was devised to yield each of the novel compounds. Different chemical reactions (including nucleophilic addition, acid-hydrolysis and amide-bond formation) and purification techniques (including vacuum filtration, liquid-liquid extractions, column chromatography and thin-layer chromatography) were required. Characterization of the compounds synthesized was accomplished through Nuclear Magnetic Resonance (NMR), High Performance Liquid Chromatography Mass Spectroscopy (HPLC-MS) and Infrared Spectroscopy (IR). The six compounds produced were then tested to determine their antimalarial activity. Should the results prove promising, they will be further investigated to develop a novel antimalarial agent that treats the disease by a mechanism distinct from those used by current treatments.

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Abstract

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Malaria, Bio-Active, Synthesis, Amide, Chalcone

Acknowledgements

Acknowledgements go to Associate Professor Clare Gutteridge of the United States Naval Academy Chemistry Department for serving as the faculty adviser for this project, to Dr. Apurba Bhattacharjee of the Walter Reed Army Institute of Research for his work with the pharmacophore modeling of the target compounds, to Mrs. Marilyn Fenwick of the USNA Chemistry Department for her administrative and logistic support, and to the USNA Trident Scholar Committee for their support and backing of this Trident project.

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Introduction

Malaria is an infectious disease spread through the bites of infected mosquitoes. It is endemic in tropical and sub-tropical regions. Malaria is caused by a tiny protozoan called Plasmodium. Specifically, there are four species which cause human disease: *Plasmodium falciparum*, *P. vivax*, *P. malariae*, and *P. ovale*. Of the four, *P. falciparum* is by far the most deadly, as well as being the one shown to have developed resistance to many anti-malarial drugs available at the present time. Despite the best efforts of medicine, strains of malaria cause over one million deaths around the world each year.

Malaria is especially widespread in areas with poor or no medical facilities such as Sub-Saharan Africa and other developing regions. Potential methods used in malaria control include: (1) the control of malaria vectors in order to prevent the spread of the disease, (2) the development of effective malaria vaccines, and (3) the development and identification of new anti-malarial chemotherapeutic agents. At present, some progress is being made in the fight against malaria vectors. In many regions where the disease is endemic, bed nets impregnated with insecticide are successfully decreasing occurrence of the disease. The use of DDT to paint the inside of houses in endemic areas has also recently been approved and proven successful. While there are no vaccines against malaria at the present time, several are in various stages of development.[1] It is likely that effective vaccines are several years away.

The greatest difficulty in controlling malaria is the rising resistance of the disease to available drugs. Of the different types of malaria, *P. falciparum* has shown the greatest potential to develop resistance to anti-malarial medications. Drug resistance can be caused by many different factors, including misuse of anti-malarial drugs, inadequate treatment length, and the

[1] Rosenthal, P.J. *Antimalarial Chemotherapy: Mechanisms of Action, Resistance, and New Directions in Drug Discovery*. Totowa, NJ, 2001; pp. 3-25.

gradual evolution of resistant strains through the long-term presence of anti-malarial drugs. In many places around the world, *P. falciparum* has become largely resistant to chloroquine. Developed during World War II, this once was the drug of choice to battle malaria across the globe. Physicians have been forced to prescribe less desirable drugs or combinations of drugs in its place. Presently, some degree or another of resistance exists to nearly all non-experimental anti-malarial drugs. Certain strains of falciparum malaria have developed which are even resistant to multiple drugs.[2]

Often new drugs are developed beginning with what is known as a lead compound. In the case of malaria, the lead compound would be a compound known to possess some anti-malarial activity already. The scientist investigating the lead then prepares compounds possessing a slightly modified structure, either by adding or subtracting functional groups in such a way as to develop a series of new compounds similar to the first. These compounds are then tested for desirable properties such as increased anti-malarial activity, increased stability in the presence of human metabolizing enzymes, or decreased toxicity. If a synthesized compound is found to have improved properties, then the process would be repeated to identify even better compounds. In this way, new and more effective drugs can be created.

The mortality of the disease itself, as well as the rapidly increasing resistance that malaria is developing to all known anti-malarial compounds, render urgency to the proposed project. New more effective drugs must be developed for the prevention and treatment of malaria before the drugs in use today completely lose their effectiveness. This important goal can be accomplished by chemists laboring to create new compounds in the laboratory, participating in projects such as this one.

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[2] Bruice, P. The Organic Chemistry of Drugs: Discovery and Design in Organic Chemistry, Prentice Hall. Upper Saddle River, NJ, **2001**.

In this project, we propose using chalcones as our lead compound series. (Chalcone is defined in the glossary at the bottom of this page). The use of this class of compound against malarial parasites is a relatively new development. Interest in chalcones arose when Licochalcone A, a natural compound obtained from the Chinese licorice root, was shown to have antimalarial properties. Synthetic analogues have subsequently been produced and determined to have potent antimalarial activity as well.[3] Although several hypotheses have been made regarding how the chalcones mediate antimalarial activity[4,5], the mechanism is not fully understood. It is clear that they work by a mechanism different from those employed by existing antimalarial drugs. This is important, since the malaria parasite will not have had the opportunity to develop resistance to the antimalarial effect of the chalcones.

Here is a brief glossary of chemical terms and an explanation of how chemical structures are typically represented:

Hybrid Structure: A combination of two separate compounds containing elements of both but identical to neither.

Chalcone: A type of organic molecule known to have antibacterial, antifungal, and anti-inflammatory properties. Chalcones have a chemical structure containing two ring structures connected by a linker portion as shown in Figure 1.

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[3] Kumar, A., Katiyar, S. B., Agarwal, A.; Chauhan, P.M.S. "Perspectives in Antimalarial Chemotherapy", *Current Medicinal Chemistry*. **2003**, 10, 1137-1150.

[4] Go, M.-L.; Liu, M.; Wilairat, P.; Rosenthal, P.J.; Saliba, K. J.; Kirk, K. "Antiparasitic Chalcones Inhibit Sorbitol-Induced Hemolysis of Plasmodium Falciparum-Infected Erythrocytes", *Antimicrobial Agents and Chemotherapy*, **2004**, 48, 3241.

[5] Ziegler, H.L.; Hansen, H. S.; Staerk, D.; Christensen, S. B.; Hgerstrand, H.; Jaroszewski, J. W. "The Antiparasitic Compound Licochalcone A Is a Potent Echinocytogenic Agent That Modifies the Erythrocyte Membrane in the Concentration Range where Antiplasmodial Activity Is Observed", *Antimicrobial Agents and Chemotherapy*, **2004**, 48, 4067.

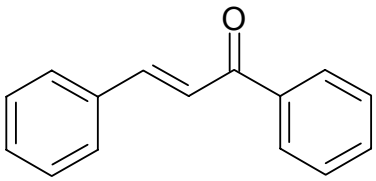

Figure 1: Basic Structure of a Chalcone

Organic chemists will often represent compounds as skeletal structures. In this representation, carbon atoms, and the corresponding hydrogen atoms connected to them are not specifically labeled and are simply drawn as the point connecting two lines as seen in Figure 2.

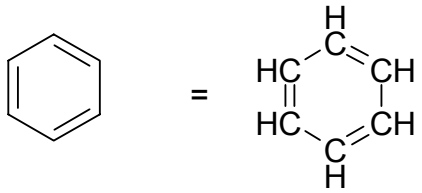

Figure 2: Equivalent Drawings of a Carbon Ring Structure

μM: A unit of concentration corresponding to 1 x 10^{-6} moles per liter.

ng/mL: A unit of concentration corresponding to 1 x 10^{-9} grams per liter.

The specific compound **1** which is the lead in this project is shown below (Figure 3). It has been found that at concentrations as low as 3 μM, this compound will inhibit growth of the malaria parasite by 50%. The concentration of a compound required to halve parasite growth is a common benchmark known as the IC_{50}. It is used to compare the antimalarial activity of different compounds; the lower the IC_{50}, the more effective a compound is as an anti-malarial. Previous studies suggest that the rings confer antimalarial properties to the compound.

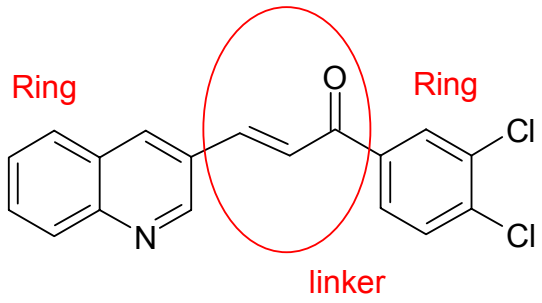

Figure 3: Compound **1**

Despite this, the compound failed to treat malaria-infected mice, probably as a result of the compound being rapidly decomposed by liver enzymes, which limited its usefulness.

A search for compounds structurally related to the chalcones, yet resistant to this metabolic destruction, led to the identification of Compound **2** (Figure 4). This compound has tremendous metabolic stability but significantly reduced antimalarial activity. (An 800 times higher concentration of this compound is required to inhibit parasite growth at a level comparable to Compound **1.**)

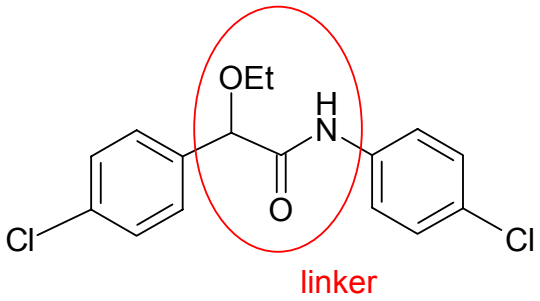

Figure 4: Compound **2**

A computer model known as a pharmacophore suggests that the antimalarial activity of Compound **1** does not depend on the linker between the rings, circled on Figure 3.[6] This project, proposes to synthesize of hybrid compounds in which the linker from Compound **2**, circled on Figure 4, connects the particular ring structures found in Compound **1**. An example of such a hybrid is Compound **3** (Figure 5). In this structure, the rings should ensure that the compound possesses antimalarial activity while the linker makes it metabolically stable.

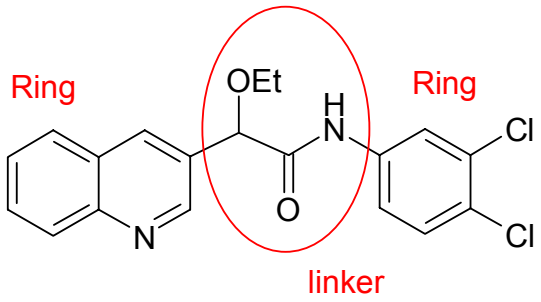

Figure 5: Compound **3**

As is the case when developing any new pharmaceutical drug, and despite the use of an effective pharmacophore model, the chances of any single compound synthesized being optimal are small. However, the probability that a desirable compound will be prepared rises considerably when a series of compounds is produced. Such a series will consist of very similar compounds that possess only slight structural variations. By systematic exploration of a range of different variations in structure, it will be possible to identify the optimal structure in the compound series.

A significant structural question is whether to have the nitrogen-containing ring on the left side of the compound, as in Compound **3**, or on the right side, as in Compound **4**.

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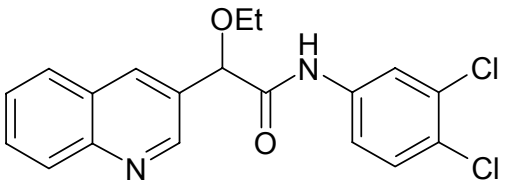
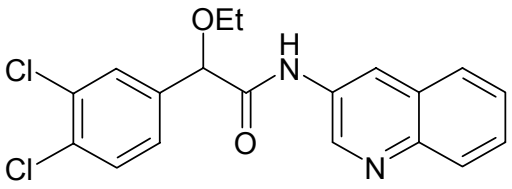
[6] Clare E. Gutteridge, Marshall M. Hoffman, Apurba K. Bhattacharjee and Lucia Gerena, "Synthesis and Antimalarial Activity of 7-Benzylamino-1-isoquinolines", *Journal of Heterocyclic Chemistry*, **2007**, 44, 633.



Compound **3**



Compound **4**

Figure 6:

Additionally, the relative placement of the two chlorines that contribute to antimalarial activity must be optimized. The final structural question concerns the location of the attachment between the linker and the nitrogen-containing ring. Therefore, it was proposed that compounds containing these variations in different combinations be assessed using pharmacophore modeling to predict likely antimalarial activity. The structures of some possible combinations are shown in Figures 7 and 8. It should be noted that each compound differs from one of the others in only one way.

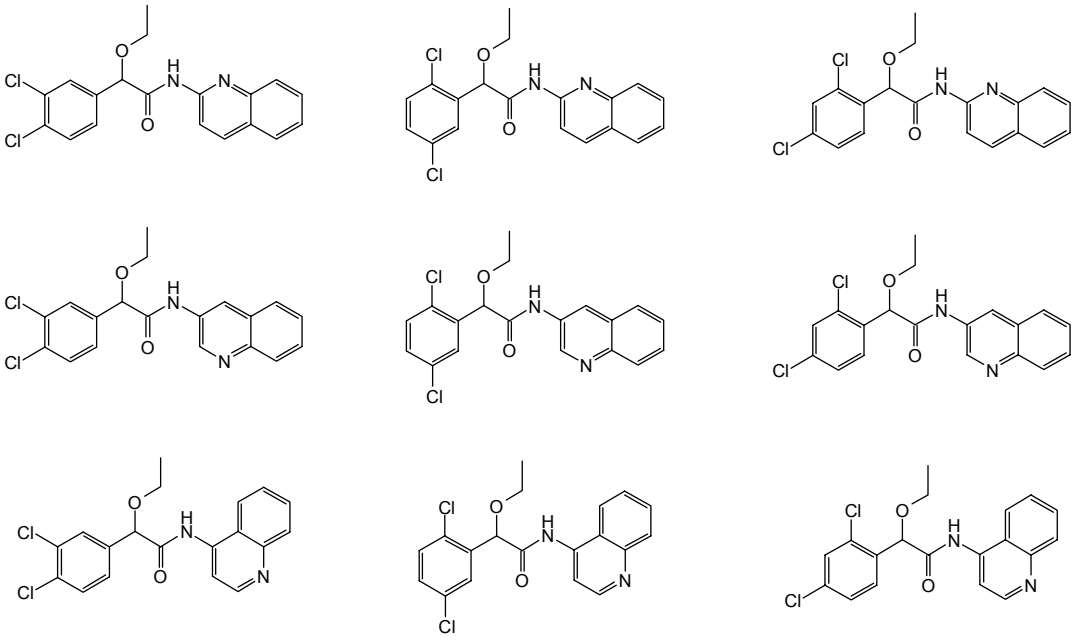


Figure 7 – Potential compounds to be synthesized (nitrogen-containing ring on the left)

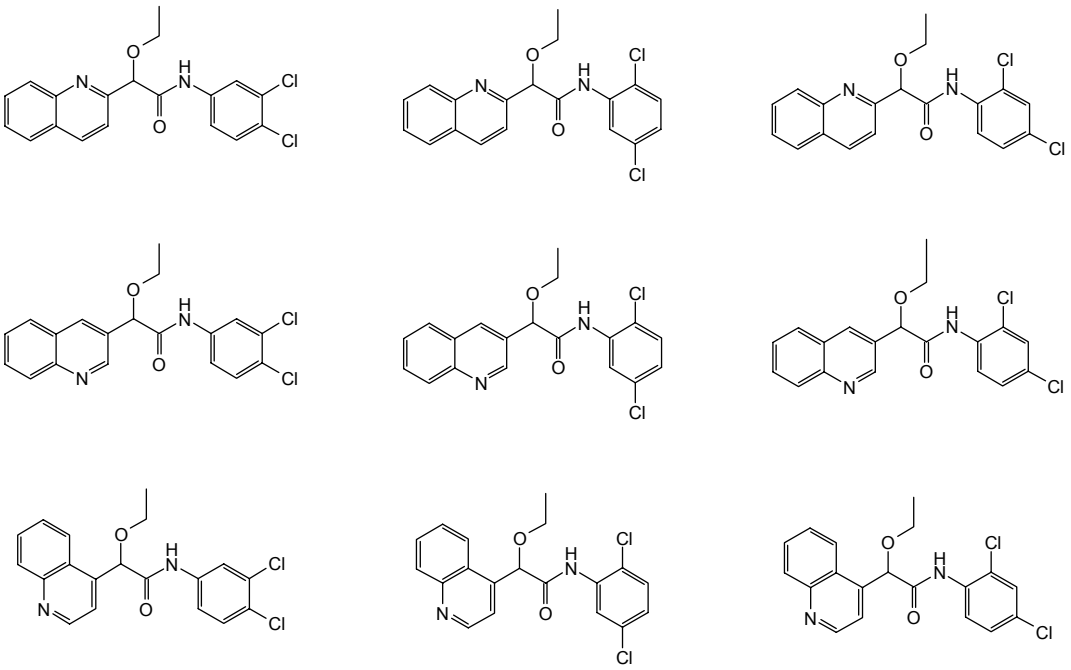


Figure 8 – Potential compounds to be synthesized (nitrogen-containing ring on the right)

In most chemical reactions, the products achieved at the conclusion of even a successful reaction include unwanted byproducts caused by side reactions occurring simultaneously with the desired reaction. Thus, exploration of methods for the purification of the reaction product was required so that the desired product in each reaction could be isolated and separated from the products of side reactions. After purification, the identity and structure of the products was confirmed: a process known as characterization. This was accomplished through the use of Nuclear Magnetic Resonance (NMR), infrared spectroscopy (IR), and High Performance Liquid Chromatography/Mass Spectroscopy (HPLC/MS).

The target compounds were prepared and characterized, and their antimalarial properties are being determined. The initial test for antimalarial activity is a determination of the concentration of compound required to inhibit parasite growth by 50%, an amount known as the IC_{50} value. Since the test is performed outside of a living organism, it is referred to as an *in vitro*

test. Such testing is complex, but is carried out routinely by specialized laboratories, such as the Walter Reed Army Institute of Research, that is testing our compounds.

Should compounds which are effective antimalarials be identified, the best will be tested for antimalarial activity in an organism, which is called an *in vivo* test. The latter test will also provide a preliminary indication as to whether the compound is resistant to the destructive process of metabolism and also whether the compound is highly toxic. *In vivo* testing will occur after the completion of this study.

Results and Discussion

This initial phase of the project involved confirmation of the design of the compounds to be prepared. To this end, eight compounds were chosen from the eighteen shown in Figures 7 and 8 to be assessed using the pharmacophore model. The structures of these eight compounds were provided to Dr. Apurba Bhattacharjee at the Walter Reed Army Institute of Research, who performed some computational modeling. The computational testing is somewhat involved, precluding computational assessment of all eighteen structures. The eight compounds to be assessed were chosen since between them they contain all the possible structural variations of the series of eighteen compounds.

The pharmacophore model searched for three specific components in the compounds it analyzed that are expected to contribute to antimalarial activity. Shown below (Figure 9) is a graphic visualization of target compound JWM9 (Figure 11) being modeled.

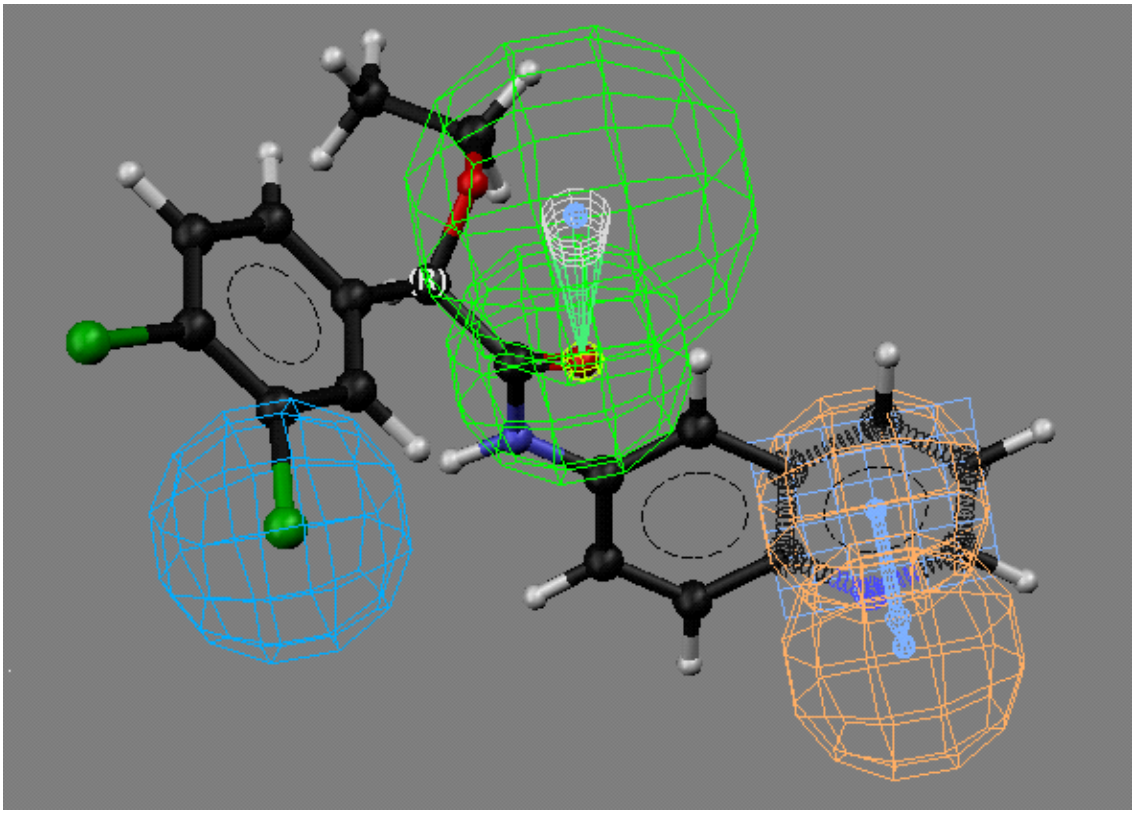


Figure 9: Pharmacophore modeling of target compound JWM9

The geometric shapes in the above graphic are the pictorial representation of the pharmacophore model. It is predicted that for a compound to be active against malaria by the chalcone-mechanism, certain functional group components must be present at those locations in space. The pharmacophore model matched portions of the newly-proposed target compounds to these shapes in the model. Accordingly, a compound with high antimalarial activity should have chemical structures which line up to all of the shapes in the model. The proposed target compounds matched the pharmacophore very closely. The structure of one of the compounds being assessed is shown in mainly in black (with chlorines in green, nitrogens in blue, oxygens in red and hydrogens in white). It can be seen that this compound has structural features that match those required by the pharmacophore and suggests that one of the chlorine atoms as well as the

nitrogen-containing ring structure will contribute to antimalarial activity. It is interesting to note that the oxygen-containing group in the linker structure is also predicted to contribute to the antimalarial activity of the compound.

In Figure 10, the structures of the first eight compounds modeled are shown, along with an associated identifying code (JWM 1-8). The number associated with each modeled structure is the IC_{50} values predicted by the computer for the compound. A lower number predicts better antimalarial properties. It can be seen by comparing values that the model predicted very little difference in antimalarial activity between the compounds tested. Had there been significant differences in predicted antimalarial activity, the most active would have been targeted for synthesis. However, the differences in the predicted IC_{50}s for the modeled structures are insignificant. For this reason, the target compounds were chosen based upon other factors.

Compounds were chosen based upon which arrangements of chlorine atoms in the chalcone series gave the best antimalarial properties[7] and upon the degree to which literature could be found describing the reactions required to synthesize the compounds.[8,9] In Figure 10, the potential compounds are divided into six series, according to how they share synthetic intermediates, and the series chosen for synthesis is highlighted (Figure 10 below). At this stage, two additional structures from the selected series were assessed in the computational model (JWM 9-10).

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[7] Clare E. Gutteridge, Daniel A. Nichols, Sean M. Curtis, Darshan S. Thota, Joseph V. Vo, Lucia Gerena, Gettayacamin Montip, Constance O. Asher, Damaris S. Diaz, Charles A. DiTusa, Kirsten S. Smith and Apurba K. Bhattacharjee, "In vitro and in vivo efficacy and in vitro metabolism of 1-phenyl-3-aryl-2-propen-1-ones against Plasmodium falciparum", *Bioorg. Med. Chem. Lett.*, **2006**, 16, 5682.

[8] Reeve, W.; Pickert, P. E. "Some α-Alkoxyarylacetic Acids", *J. Am. Chem. Soc.*, **1957**; 79, 1932.

[9] Quan, M. L.; Wityak, J.; Dominguez, C.; Duncia, J. V.; Kettner, C. A.; Ellis, C. D.; Liauw, A. Y.; Park, J. M.; Santella, J. B.; Knabb, R. M.; Thoolen, M. J.; Weber, P. C.; Wexler, R. R. "Biaryl substituted alkylboronate esters as thrombin inhibitors", *Bioorg. Med. Chem. Lett.*, **1997**, 7, 1595; Quan, M. L.; Pruitt, J. R.; Ellis, C. D.; Liauw, A. Y.; Galemmo, R. A.; Stouten, P. F. W. "Bisbenzamidine isoxazoline derivatives as factor Xa inhibitors", *Bioorg. Med. Chem. Lett.*, **1997**, 7, 2813.

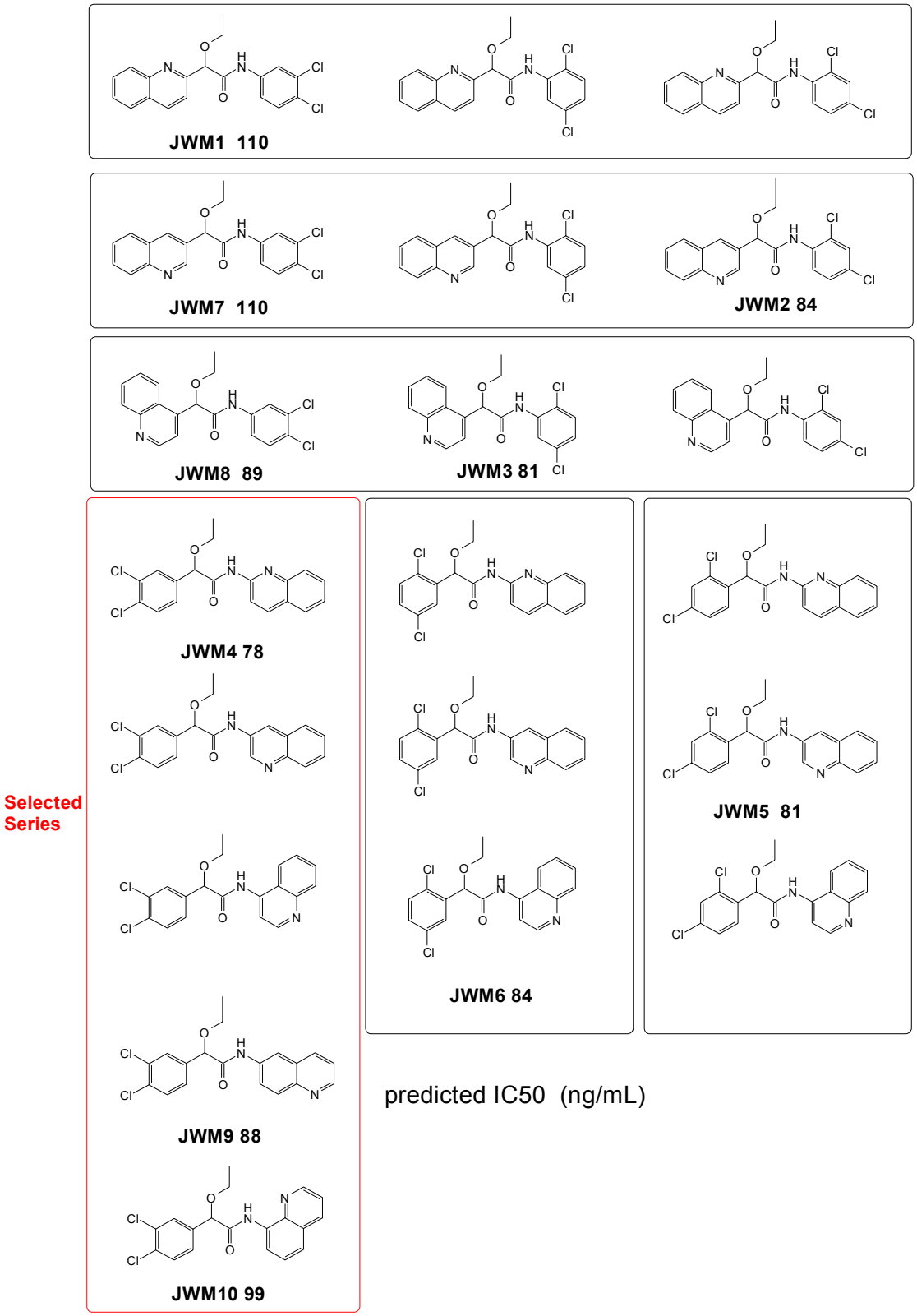

Figure 10: Results of pharmacophore modeling

The six target compounds ultimately chosen for synthesis are shown in the box in Figure 11. Also shown is the synthetic scheme which was developed for synthesis of this series. Each compound is accessible by a four-step synthetic pathway, with the first three steps in common. The arrows represent reactions performed to change one compound into the next. Beside the arrows are shown the specific chemical reagents required to carry out each of these reactions. The final step of the scheme is unique for each of the six target compounds.

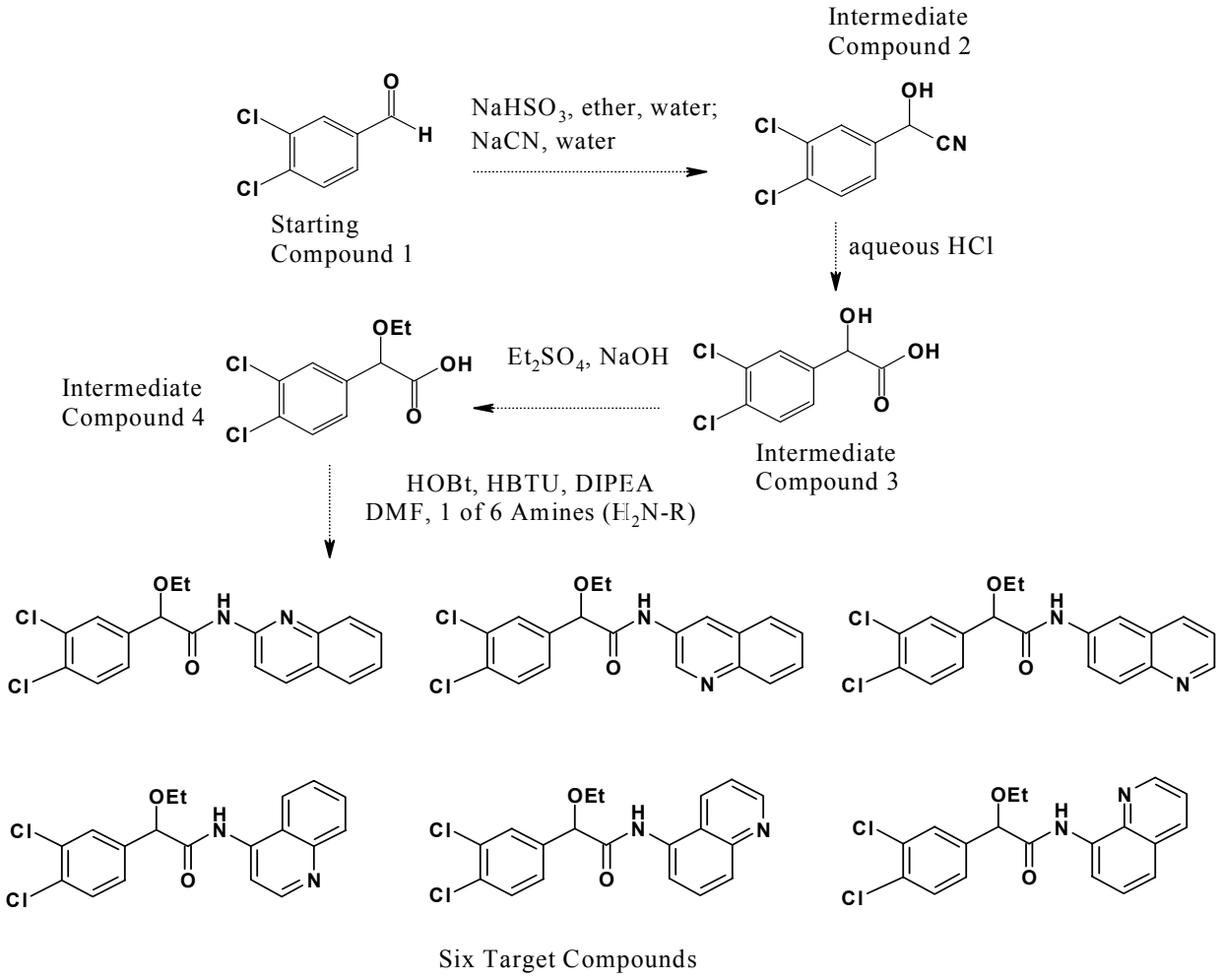

Figure 11: Synthetic scheme for the six target compounds

In this research, **Intermediate Compounds 2**, **3**, and **4** have been synthesized as well as **Target Compounds 1** through **6**. Characterization of these compounds has also been completed. The six target compounds have been sent to WRAIR for *in vitro* antimalarial testing.

Experimental Section

Synthesis of the six novel compounds began with the commercially available starting material **Starting Compound 1**, 3,4-dichlorobenzaldehyde. This section provides a description of the reactions which were used to produce **Target Compounds 1** through **6** (Figure 12) by way of a four-step synthesis (Figure 11). The conditions by which the reactions were performed varied. Reactions were performed in ice, on a hot plate, or under ambient conditions. The time required for specific steps also varied from almost instantaneous to stirring overnight or even for several days. Most steps of the process were performed several times, some as many as six times, and with varying amounts of starting material. Each step was normally performed by initially using only a small amount of starting material, in order to ensure that the reaction could be performed successfully. Upon successful completion, the reaction scale would then be increased.

After each step, leftover reagents and compounds created through side reactions might have been present along with the desired product. Purification removed these impurities. Several different purification techniques were used, most commonly column chromatography and liquid-liquid extraction, which were used extensively. Liquid-liquid extraction works by separating compounds based upon their differing solubility in organic and aqueous solvents. Column chromatography works by separating compounds through their differences in polarity. In most cases, both techniques were used to achieve the greatest purity. The process had to start

with a significant initial amount of starting material since the yield of each step was never 100%, so less material was available for later steps. Only 10mg of the desired compound is needed to perform in-vitro antimalarial testing. However, additional compound was needed to perform the tests necessary for characterization of the product. A more detailed description of the laboratory work is provided in Appendix A.

Characterization

Characterization of the compounds was performed through Nuclear Magnetic Resonance spectroscopy (NMR), mainly ^{1}H-NMR but also through ^{13}C-NMR and COSY NMR which is a more complex ^{1}H-NMR technique. Infrared (IR) spectroscopy and High Performance Liquid Chromatography Mass Spectroscopy (HPLC-MS) were also used.

NMR measures the magnetic field created by spinning nuclei and their interactions with one another. This magnetic field can be forced to align along a specific axis if placed in an extremely strong magnetic field such as that of the NMR instrument. While NMR can be used to study any element possessing nuclear spin, for an organic chemist and in this project, only ^{1}H and ^{13}C nuclei were explored. The NMR instrument measures radio frequencies emitted as the nuclei drop from a higher to a lower spin state.

The signal is analyzed using Fourier Transformation, creating the final NMR spectrum. This spectrum can be affected by multiple structural features within the compound. The chemical environment, in particular proximity to nearby spinning nuclei, greatly affects the appearance of peak signals. The relative size of peak signals can be used to determine how many nuclei it represents in comparison to other peaks. For example, in a ^{1}H spectrum, the area under a peak representing a CH_2 is 2/3 of that under the peak representing a CH_3. The peak appearance

is made more complex by the fact that proximity to non-identical nuclei causes splitting in the peaks signals (one neighboring nucleus splits a "singlet" peak into a "doublet"; two neighbors splits a "singlet" peak into a "triplet" and so on).

The location of the peak is also important. Peak location depends on nuclear shielding, in particular the magnetic field strength that the atom feels. Certain functional groups cause dramatic changes in shielding. Nearness to an electron withdrawing group causes the nuclei to experience a greater magnetic field and thus forces a peak to appear further downfield or further to the left-hand side of the spectrum. Electron donating groups would have the opposite effect. It is these differences in peak splitting, integration and position which allow NMR to be so useful in determining the structure of chemical compounds.

The structures of these novel compounds was analyzed first using ^{1}H NMR to determine if all desired peaks were present in the expected position and with the expected splitting pattern. This analysis was adequate to determine and positively identify that the compound produced was in fact the desired structure. Novel compounds were also analyzed using ^{13}C and COSY NMR techniques, IR spectroscopy and HPLC mass spectrometry in order to correlate peaks to specific hydrogen and carbon atoms, to further characterize the structure of the compounds, and to support the conclusion that the correct compound had been made.

Antimalarial Results

All six novel compounds synthesized in this research were submitted to the Walter Reed Army Institute of Research (WRAIR) for in-vitro antimalarial testing in March 2008. This testing could not be done at the Naval Academy because it requires specialized training and equipment.

Results for the first four novel compounds produced are shown in Figure 12 (testing of the last two compounds is on-going); under the corresponding structure, are the *in vitro* anti-malarial activities, expressed as IC_{50}s. The fifth structure included in Figure 12 is the best chalcone synthesized to date.

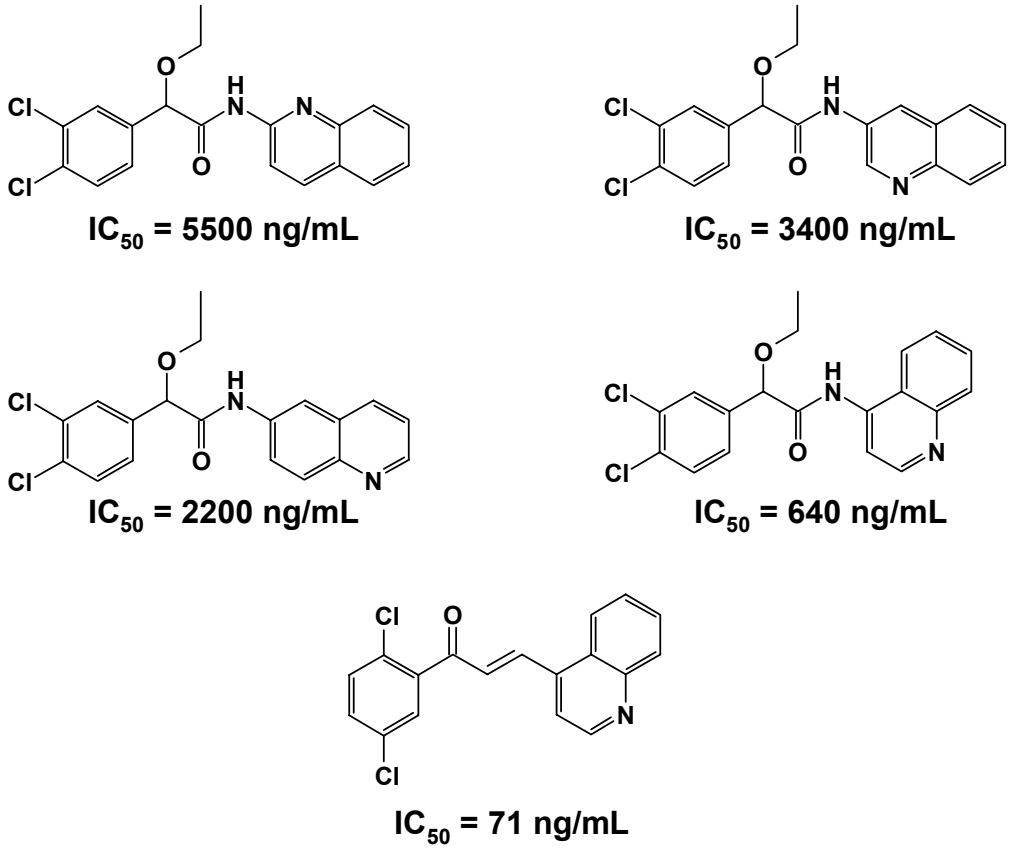

Figure 12: *In vitro* anti-malarial activity results

With an IC_{50} of 640 ng/mL, the most active of the four novel compounds, has an activity within ten-fold of the best chalcone (IC_{50} of 71 ng/mL), and within twenty-fold of the threshold for a compound to be selected for pre-clinical assessment by Walter Reed (threshold IC_{50} is 30

ng/mL). Considering that the latter compound is the result of over a decade of investigation, to have prepared compounds so close in activity suggests that with further optimization the novel series may well yield a candidate for pre-clinical assessment. Furthermore, the significant variation in IC_{50} for the four novel compounds suggests that relatively small structural changes will result in significant changes in activity, so compounds with improved activity should be accessible to those who continue this project.

With an IC_{50} of 640 ng/mL, the most active of the four novel compounds has significant enough *in vitro* anti-malarial activity to warrant further *in vivo* testing. This will determine whether the compound is able to treat a mouse infected with malaria. This will require preparation of a larger quantity of compound, which will be submitted to collaborators of the Walter Reed Army Institute of Research. Toxicity testing may also be performed to determine if the compound can be safely used in a living organism.

It should be noted that the actual *in vitro* IC_{50} results are somewhat higher than those predicted by the pharmacophore. Additionally, the variance in activity between the different compounds was not predicted by the pharmacophore. The pharmacophore is a model, and as such, can be improved with novel results. Thus, the results obtained herein could be incorporated into the pharmacophore to improve its ability to predict antimalarial activities.

Conclusion

This research produced a series of novel organic compounds which possess antimalarial activity. The best activity achieved was within just 10-fold of that of the best chalcone, quite an accomplishment upon synthesis of six compounds. The large variance in the activities of the tested compounds suggests that changes in chemical structure have a significant effect on the

anti-malarial activity. This will enable further optimization of the compound structure for future work.

The compound with IC_{50} of 640 ng/mL has significant enough *in vitro* anti-malarial activity to warrant further testing. The next step should be *in vivo* testing, which will determine whether the compound is able to treat a mouse infected with malaria.

Development of the synthetic pathways used successfully to synthesize the target compounds brings benefit. All experiments conducted throughout this research have been extensively recorded for future reference. Synthetic schemes, notes on reactions, amounts of reagents, reaction conditions and purification methods used are documented. Thus, clear directions are available to others who may wish to synthesize these, or related compounds.

The research performed will have a significant impact on antimalarial research as well as research into other areas of medicinal chemistry. Possible directions for future research include increasing the efficiency of the reactions, in-vivo, metabolic stability and toxicity testing of the compounds already prepared, and synthesis of additional compounds directed by the antimalarial results of the initial series of compounds. It is therefore possible that the research performed could help pave the way for a new, more effective antimalarial drug.

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Appendix A: Technical Procedures

Preparation of Intermediate Compound 1

The synthesis began with 6.0 grams of commercially available 3,4-dichlorobenzaldehyde (**Starting Compound 1**), which was combined with 39.0mL of ether. A second solution was made from 10.0g of $NaHSO_3$ and 15.0mL of water. The bisulfite solution was added to the benzaldehyde solution, and the resulting mixture was mixed well and then put on ice for 10 minutes. Small, clear crystals formed as the solution cooled. As the solution was allowed to warm up to room temperature, it was shaken vigorously, which caused the crystals to transform into a large amount of bisulfite addition product, a less dense white powder. This was recovered by filtration under vacuum, yielding 19.2g. It was added to 235mL of water then combined with a solution of 5.8821g of NaCN in 24.0mL water, which resulted in the immediate formation of a white precipitate.

An attempt was made to remove this precipitate via vacuum filtration, but this failed due to the precipitate transforming into an oil upon standing. Therefore, the oil was extracted into diethyl ether. The ether layer was separated from the aqueous layer, then moisture was removed using $MgSO_4$. The ether was removed by evaporation under reduced pressure, leaving **Intermediate Compound 1** (JWM702) as a yellowish oily product (18.5g, 53% yield from **Starting Compound 1**, 3,4-dichlorobenzaldehyde). The reaction was performed on a larger scale once the small scale was shown to be effective. It was run four times, and the product was identified and characterized through ^{1}H NMR. ^{1}H NMR (deuteriochloroform): δ 7.61(1H, d); 7.50(1H, d); 7.34(1H, dd); 5.50(1H, s).

Preparation of Intermediate Compound 2

50mL of concentrated HCl was added to **Intermediate Compound 1** and it was stirred at 90°C overnight. After thirteen hours, the solution was allowed to cool to room temperature. The HCl(aq.) was removed with a pipette. 25mL of ether and 3mL of saturated NaCl were added; then the ether layer was separated and dried over anhydrous $MgSO_4$. Following filtration, the ether was removed by evaporation under reduced pressure, leaving **Intermediate Compound 2** (JWM716) as a reddish oil (12.5g, 76% yield from **Intermediate Compound 1**). The reaction was performed on a larger scale once the small scale was shown to be effective. It was run four times, and the product was identified and characterized through ^{1}H NMR. ^{1}H NMR (deuteriochloroform): δ 7.54(1H, d); 7.42(1H, d); 7.28(1H, dd); 5.18(1H, s).

Preparation of Intermediate Compound 3

A solution containing 35.82g of NaOH and 118.8mL of water was warmed to 90°C. **Intermediate Compound 2** was added, followed by 52.5mL of Et_2SO_4, which was slowly added over 2 hr. The resulting solution was then stirred at 90°C overnight. The solution was then allowed to cool to room temperature. It was then acidified with conc. HCl, adding dropwise until the pH was approximately 1, which required 30mL. The organic product was extracted into benzene, resulting in a solution of a dark gold color. 10mL of saturated NaCl was added, and after separation the benzene layer was dried over anhydrous $MgSO_4$. The benzene was removed by evaporation under reduced pressure, leaving the oily product. The oil was dried under vacuum overnight to remove residual benzene. Through IR and NMR spectroscopy, the oily product was determined to be a combination of unreacted starting material and the desired product. The oil was then purified by silica column chromatography, using a column with a

width of approximately 45mm. The solvent used was 15% ethyl acetate and 85% hexanes. To prepare the column, two columns worth of solvent were run through the silica. The product, dissolved into dichloromethane, was loaded onto a small additional amount of silica gel. This was then added to the column. After eluting the column, the product containing fractions were combined and the solvent evaporated under reduced pressure, leaving **Intermediate Compound 3** (JWM726B) as a yellowish oil (4.2g, 26% yield from **Intermediate Compound 2**). The reaction was performed on a larger scale once the small scale was shown to be effective. This product, **Intermediate Compound 3**, is the final common product between the six target compounds. It was run four times, and the product was identified and characterized through ^{1}H NMR. ^{1}H NMR (deuteriochloroform): δ 7.55(1H, d); 7.44(1H, d); 7.29(1H, dd); 4.83(1H, s); 3.66-3.50 (2H, m); 1.31-1.26 (3H, t).

Preparation of Target Compound 1

100mg of **Intermediate Compound 3** was added to 3mL of *N,N*-dimethylformamide (DMF). 0.228mL of *N,N*-diisopropylethylamine (DIPEA) was added to the solution, followed by 0.0729g of 2-aminoquinoline. 0.0749g of 1-hydroxybenzotriazole (HOBt) was added to the solution followed by 0.1743g of *O*-(benzotriazol-1-yl*)-N,N,N′,N′*-tetramethyluronium hexafluorophosphate (HBTU). This solution was allowed to stir overnight, diluted with ethyl acetate, and then washed with water to remove the DMF. 1mL of saturated NaCl was added; then the layers were separated. The ethyl acetate layer was dried over anhydrous $MgSO_4$; then the solvent was the removed by evaporation under reduced pressure. The residue was purified on a silica column, starting with 10% ethyl acetate and 90% hexanes as the solvent. The solvent polarity was progressively increased up to 40% ethyl acetate and 60% hexanes. The fractions

which contained product were collected and the solvent evaporated to give **Target Compound 1** (JWM734A) as an off-white solid (110mg, 72% yield from **Intermediate Compound 3**). The reaction was run once, and the product was identified and characterized through ^{1}H, ^{13}C and COSY NMR spectroscopy, HPLC mass spectroscopy and IR spectroscopy. ir: 3456, 3000, 1742, 1638, 1538, 1465, 1355, 1312, 1133 cm^{-1}; ^{1}H NMR (deuteriochloroform): δ 9.33(1H, s); 8.37(1H, d); 8.16(1H, d); 7.88(1H, d); 7.78(1H, d); 7.68(1H, t); 7.62(1H, s); 7.49-7.43(2H, m); 7.37(1H, dd); 4.86(1H, s); 3.70-3.62(2H, m); 1.37(3H, t); ^{13}C NMR (deuteriochloroform): δ 169.1, 150.2, 146.7, 138.8, 137.3, 133.0, 132.9, 130.7, 130.3, 128.9, 127.7, 127.6, 126.6, 126.4, 125.5, 114.1, 81.0, 66.2, 15.3. The ^{1}H NMR spectrum for **Target Compound 1** is shown below in Figure 13.

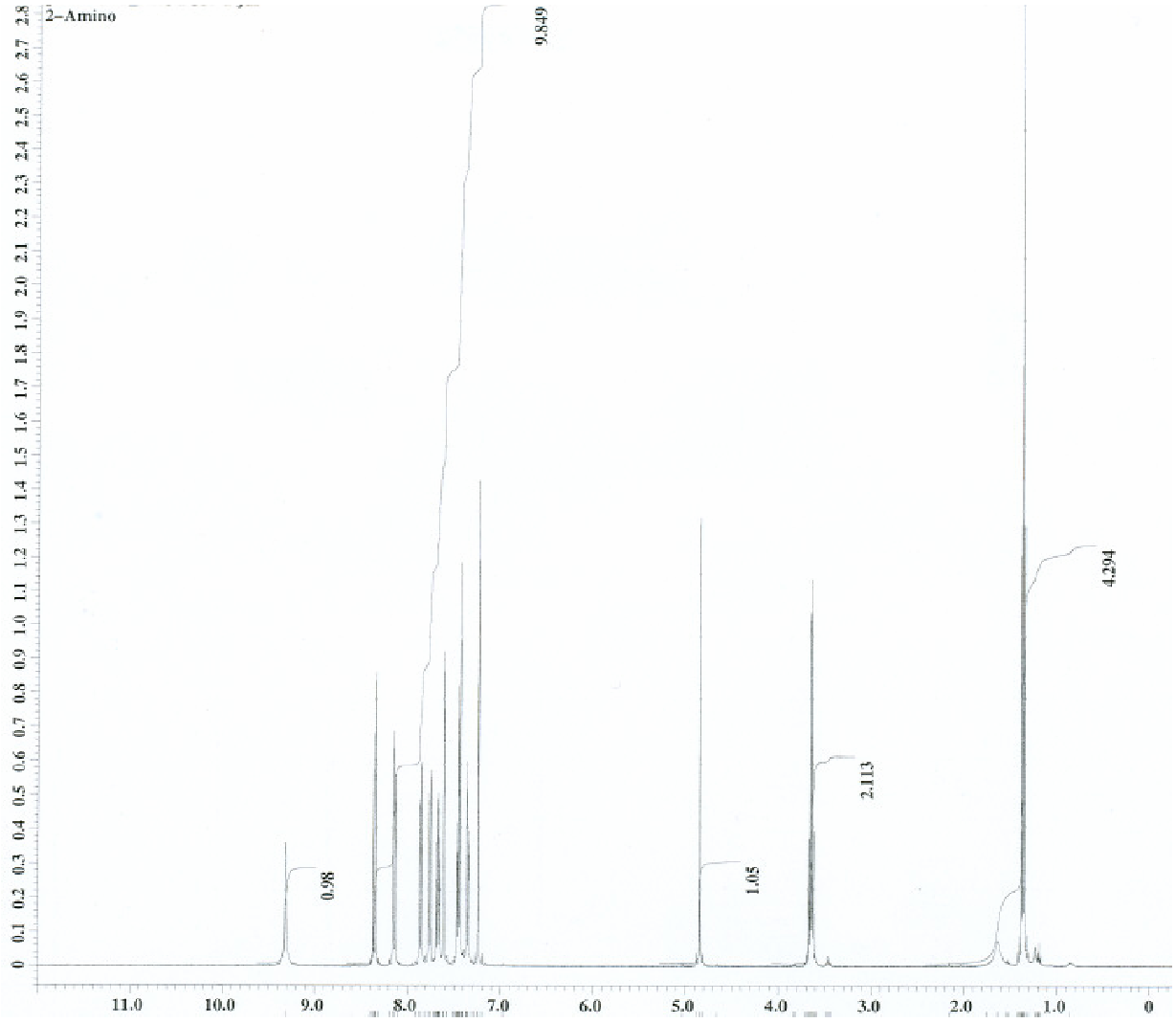

Figure 13: ^{1}H NMR spectrum of **Target Compound 1**

Preparation of Target Compound 2

105mg of **Intermediate Compound 3** was added to 3mL *N,N*-dimethylformamide (DMF). 0.228mL of *N,N*-diisopropylethylamine (DIPEA) was added to the solution, followed by 0.0751g of 6-aminoquinoline. 0.0755g of 1-hydroxybenzotriazole (HOBt) was added to the solution, followed by 0.1751g of *O*-(benzotriazol-1-yl*)-N,N,N′,N′*-tetramethyluronium

hexafluorophosphate (HBTU). This solution was allowed to stir overnight, diluted with ethyl acetate and then washed with water to remove the DMF. 1mL of saturated NaCl was added; then the layers were separated. The ethyl acetate layer was dried over anhydrous $MgSO_4$; then the solvent was the removed by evaporation under reduced pressure. The residue was purified on a silica column, starting with 10% ethyl acetate and 90% hexanes as the solvent. The solvent polarity was progressively increased up to 100% ethyl acetate. The fractions which contained product were collected and the solvent evaporated to give **Target Compound 2** (JWM741A) as a yellow solid (99.7mg, 63% yield from **Intermediate Compound 3**). The reaction was run once, and the product was identified and characterized through ^{1}H, ^{13}C and COSY NMR spectroscopy, HPLC mass spectroscopy and IR spectroscopy. ir: 3452, 3200, 1727, 1543, 1502, 1469, 1413, 1358, 1225, 1133 cm^{-1}; ^{1}H NMR (deuteriochloroform): δ 8.84 (1H, dd); 8.77(1H, s); 8.46 (1H, s); 8.10(1H, d); 8.06(1H, d); 7.64-7.59 (2H, m); 7.47 (1H, d); 7.40-7.34 (2H, d); 4.86 (1H, s); 3.72-3.63 (2H, m); 1.37 (3H, t); ^{13}C NMR (deuteriochloroform): δ 168.3, 149.7, 145.7, 137.3, 136.1, 134.9, 133.0, 132.9, 130.7, 130.4, 128.8, 126.4, 123.1, 121.9, 116.3, 80.8, 66.2, 15.3. The ^{1}H NMR spectrum for **Target Compound 2** is shown below in Figure 14.

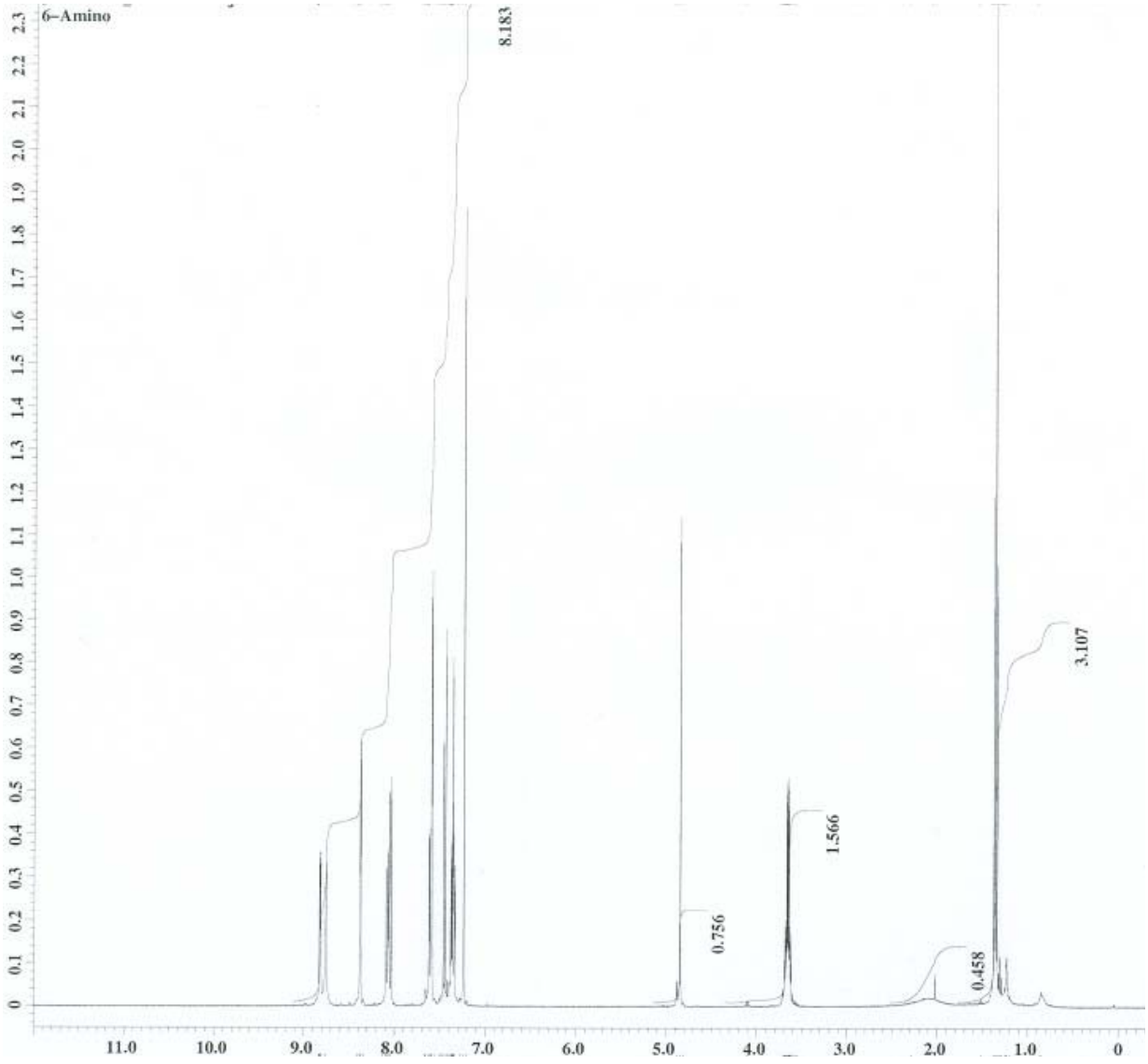

Figure 14: ^{1}H NMR spectrum of **Target Compound 2**

Preparation of Target Compound 3

103mg of **Intermediate Compound 3** was added to 3mL *N,N*-dimethylformamide (DMF). 0.231mL of *N,N*-diisopropylethylamine (DIPEA) was added to the solution, followed by 0.0743g of 3-aminoquinoline. 0.0780g of 1-hydroxybenzotriazole (HOBt) was added to the solution, followed by 0.1770g of *O*-(benzotriazol-1-yl*)-N,N,N′,N′*-tetramethyluronium hexafluorophosphate (HBTU). This solution was allowed to stir for two days, diluted with ethyl

acetate and washed with water to remove the DMF. 1mL of saturated NaCl was added; then the layers were separated. The ethyl acetate layer was dried over anhydrous $MgSO_4$; then the solvent was the removed by evaporation under reduced pressure. The residue was purified on a silica column, starting with 10% ethyl acetate and 90% hexanes as the solvent. The solvent polarity was progressively increased up to 100% ethyl acetate. The fractions containing product were collected and the solvent evaporated to give **Target Compound 3** (JWM755A) as an off-white solid (96.2mg, 62% yield from **Intermediate Compound 3**). The reaction was run once, and the product was identified and characterized through ^{1}H, ^{13}C and COSY NMR spectroscopy, HPLC mass spectroscopy and IR spectroscopy. ir: 3467, 3323, 3141, 3055, 2999, 1721, 1569, 1506, 1409, 1228, 1125 cm^{-1}; ^{1}H NMR (deuteriochloroform): δ 8.86-8.76(3H, m); 8.04(1H, d); 7.76(1H, d); 7.66-7.59(2H, m); 7.56-7.45(2H, m); 7.36(1H, dd); 4.88(1H, s); 3.73-3.64(2H, m); 1.38(3H, t); ^{13}C NMR (deuteriochloroform): δ 168.7, 145.5, 143.9, 137.1, 133.1, 133.0, 130.8, 130.6, 129.2, 128.9, 128.6, 128.1, 127.9, 126.4, 124.0, 80.7, 66.2, 15.4. The ^{1}H NMR spectrum for **Target Compound 3** is shown below in Figure 15.

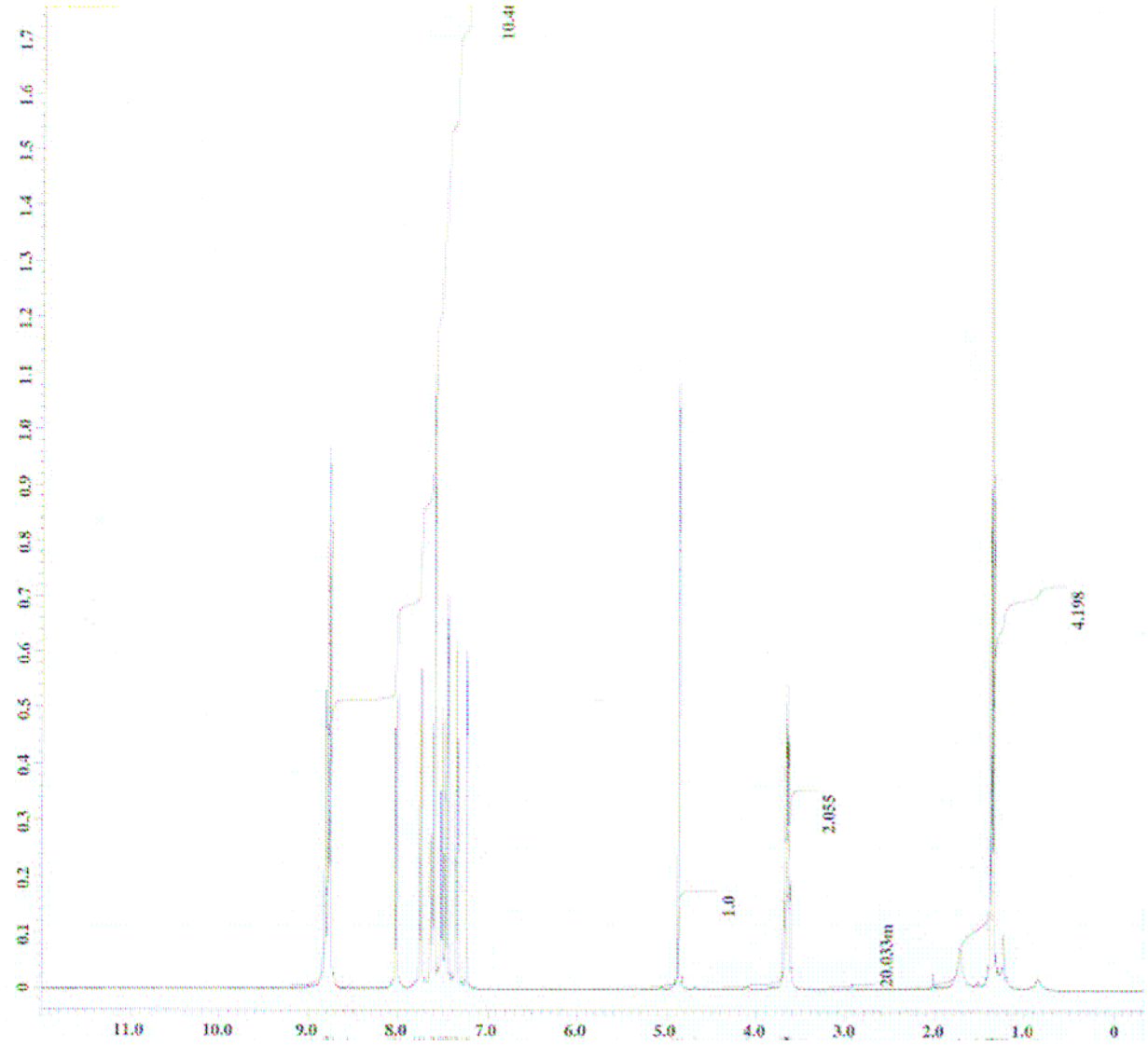

Figure 15: ^{1}H NMR spectrum of **Target Compound 3**

Preparation of Target Compound 4

107mg of **Intermediate Compound 3** was added to 3mL *N,N*-dimethylformamide (DMF). 0.230mL of *N,N*-diisopropylethylamine (DIPEA) was added to the solution, followed by 0.0745g of 4-aminoquinoline. 0.0780g of 1-hydroxybenzotriazole (HOBt) was added to the solution, followed by 0.1781g of *O*-(benzotriazol-1-yl*)-N,N,N′,N′*-tetramethyluronium hexafluorophosphate (HBTU). This solution was allowed to stir for two days, diluted with ethyl

acetate and washed with water to remove the DMF. 1mL of saturated NaCl was added; then the layers were separated. The ethyl acetate layer was dried over anhydrous $MgSO_4$; then the solvent was the removed by evaporation under reduced pressure. The residue was purified on a silica column, starting with 10% ethyl acetate and 90% hexanes as the solvent. The solvent polarity was progressively increased up to 80% ethyl acetate and 20% hexanes. The product containing fractions were collected and the solvent evaporated to give **Target Compound 4** (JWM758) as a pale orange solid (36.7mg, 23% yield from **Intermediate Compound 3**). The reaction was run once, and the product was identified and characterized through ^{1}H, ^{13}C and COSY NMR spectroscopy, HPLC mass spectroscopy and IR spectroscopy. ir: 3470, 3180, 1748, 1563, 1423, 1350, 1302, 1225, 1120 cm^{-1}; ^{1}H NMR (deuteriochloroform): δ 9.60(1H, s); 8.84(1H, d); 8.28(1H, d); 8.13(1H, d); 7.81(1H, d); 7.75(1H, t); 7.66-7.60(2H, m); 7.47(1H, d); 7.36(1H, d); 4.93(1H, s); 3.79-3.66(2H, m); 1.44(3H, t); ^{13}C NMR (deuteriochloroform): δ 168.5, 151.4, 148.9, 139.4, 136.7, 133.1, 130.9, 129.6, 128.9, 126.9, 126.4, 119.9, 118.7, 110.3, 81.2, 66.4, 15.5. The ^{1}H NMR spectrum for **Target Compound 4** is shown in Figure 16 below.

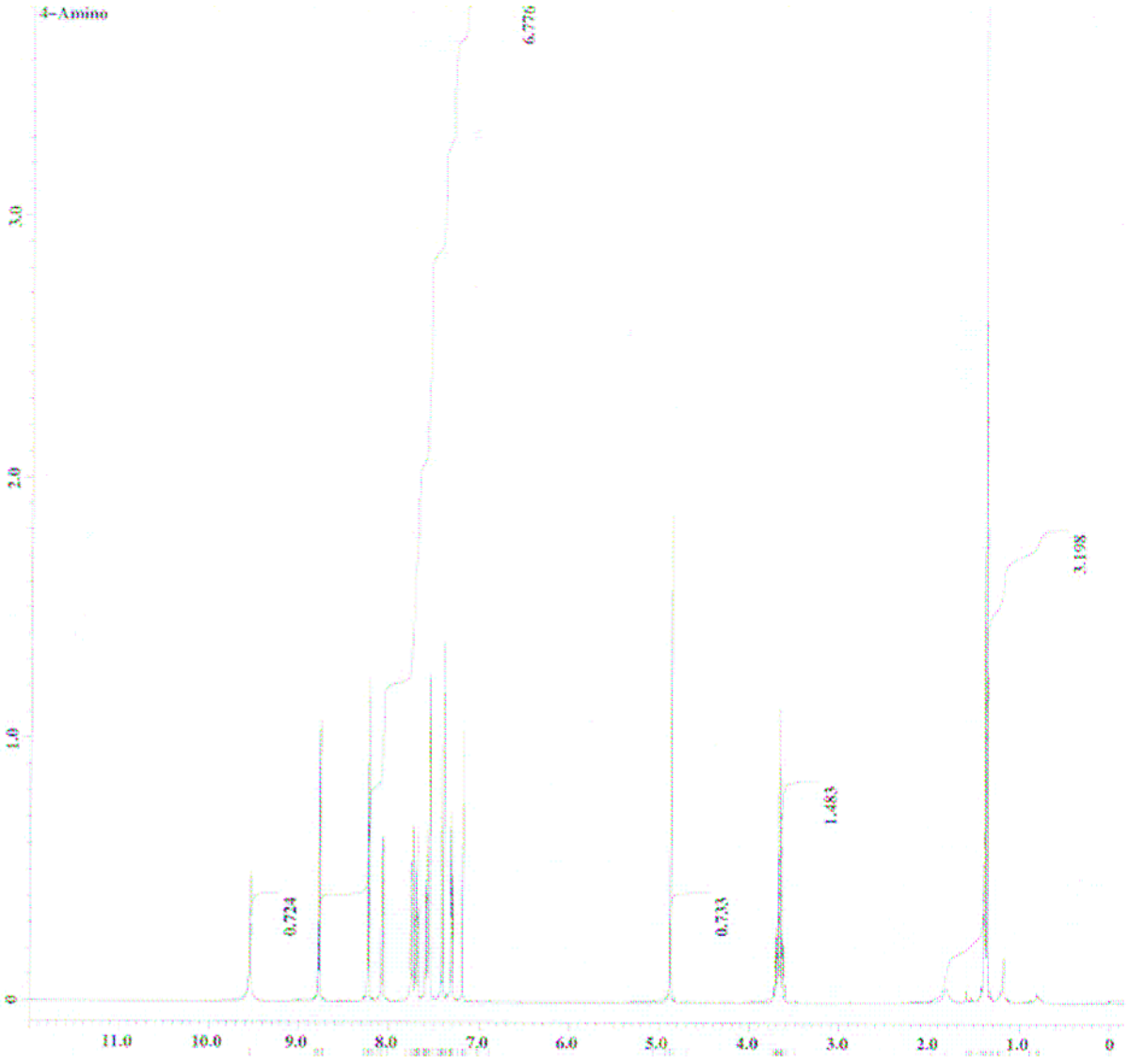

Figure 16: ^{1}H NMR spectrum of **Target Compound 4**

Preparation of Target Compound 5

105mg of **Intermediate Compound 3** was added to 3mL *N,N*-dimethylformamide (DMF). 0.230mL of *N,N*-diisopropylethylamine (DIPEA) was added to the solution, followed by 0.0751g of 5-aminoquinoline. 0.0783g of 1-hydroxybenzotriazole (HOBt) was added to the solution, followed by 0.1772g of *O*-(benzotriazol-1-yl)-*N,N,N′,N′*-tetramethyluronium hexafluorophosphate (HBTU). This solution was allowed to stir overnight, diluted with ethyl

acetate and washed with water to remove the DMF. 1mL of saturated NaCl was added; then the layers were separated. The ethyl acetate layer was dried over anhydrous $MgSO_4$; then the solvent was the removed by evaporation under reduced pressure. The residue was purified on a silica column starting with 0.5% methanol and 99.5% dichloromethane as the solvent. The solvent polarity was progressively increased up to 2% methanol and 98% dichloromethane. The fractions containing product were collected and the solvent evaporated to give **Target Compound 5** (JWM766A) as a yellowish oil (17.3mg, 11% yield from **Intermediate Compound 3**). The reaction was run once, and the product was identified and characterized through ^{1}H, ^{13}C and COSY NMR spectroscopy. ^{1}H NMR (deuteriochloroform): δ 9.08(1H, s); 8.95(1H, s); 8.11(1H, d); 8.02(1H, d); 7.96(1H, d); 7.69(1H, t); 7.64(1H,s); 7.51-7.38(3H,m); 4.94(1H, s); 3.77-3.68(2H, m); 1.42(3H, t); ^{13}C NMR (deuteriochloroform): δ 168.5, 150.6, 148.7, 137.3, 133.1, 132.9, 131.5, 130.8, 129.5, 129.1, 128.8, 127.4, 126.3, 122.1, 121.3, 120.5, 81.1, 66.3, 15.5. The ^{1}H NMR spectrum for **Target Compound 5** is shown in Figure 17 below.

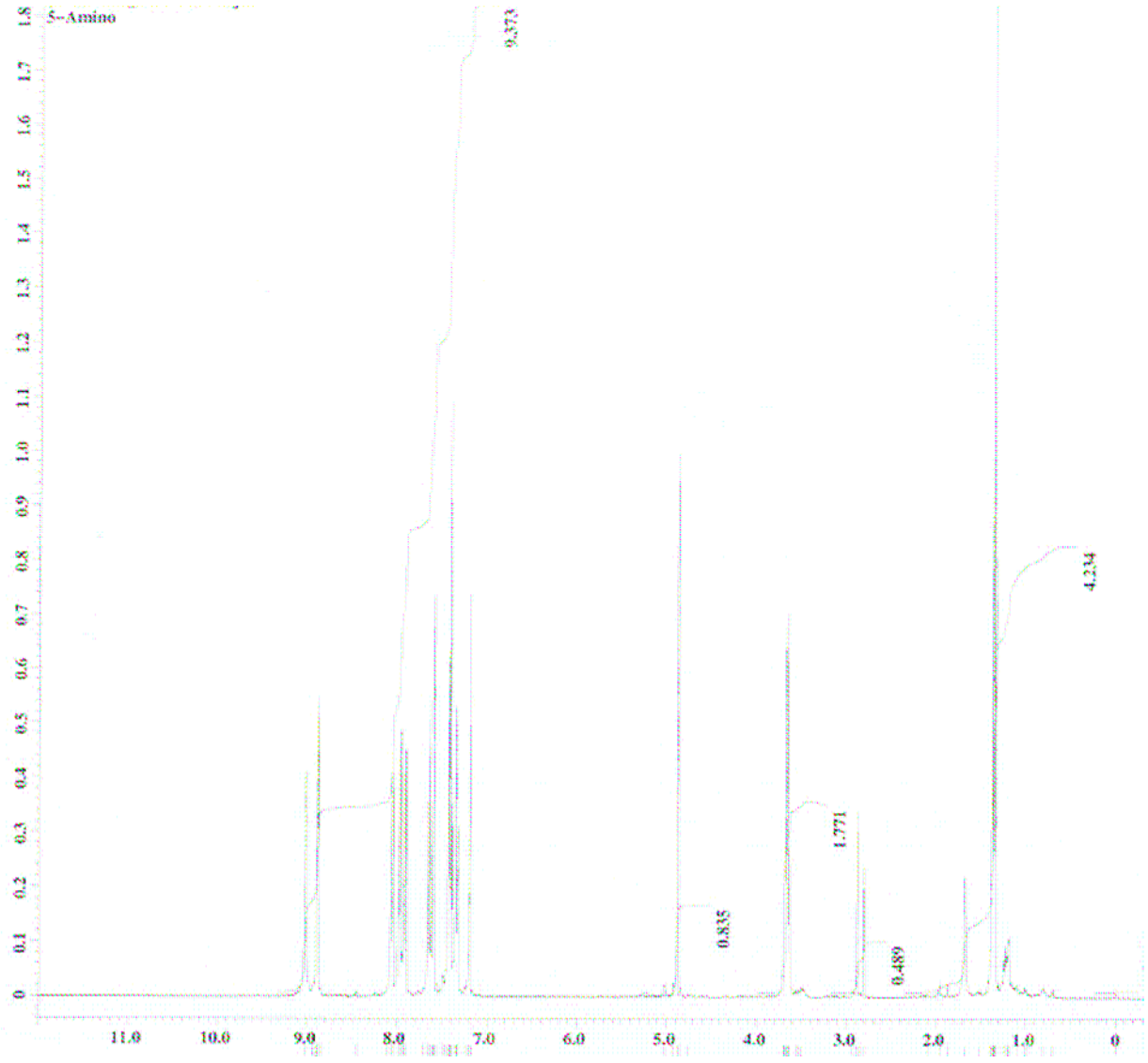

Figure 17: ^{1}H NMR spectrum of **Target Compound 5**

Preparation of Target Compound 6

146mg of **Intermediate Compound 3** was added to 4.5mL *N,N*-dimethylformamide (DMF). 0.340mL of *N,N*-diisopropylethylamine (DIPEA) was added to the solution, followed by 0.1143g of 8-aminoquinoline. 0.1134g of 1-hydroxybenzotriazole (HOBt) was added to the solution, followed by 0.2583g of *O*-(benzotriazol-1-yl)-*N,N,N′,N′*-tetramethyluronium

hexafluorophosphate (HBTU). This solution was allowed to stir overnight, diluted with ethyl acetate and washed with water to remove the DMF. 1mL of saturated NaCl was added; then the layers were separated. The ethyl acetate layer was dried over anhydrous $MgSO_4$; then the solvent was the removed by evaporation under reduced pressure. The residue was purified on a silica column with dichloromethane as the solvent. The fractions which contained product were collected and the solvent evaporated to give **Target Compound 6** (JWM752) as a white solid (90mg, 41% yield from **Intermediate Compound 3**). The reaction was run once, and the product was identified and characterized through ^{1}H, ^{13}C and COSY NMR spectroscopy, HPLC mass spectroscopy and IR spectroscopy. ir: 3425, 3053, 1719, 1571, 1514, 1428, 1357, 1101, 1059 cm^{-1}; ^{1}H NMR (deuteriochloroform): δ 11.10(1H, s); 8.88(1H, dd); 8.70(1H, dd); 8.16(1H, dd); 7.70(1H, s); 7.55-7.43(5H, m); 4.90(1H, s); 3.74(2H, q); 1.45(3H, t); ^{13}C NMR (deuteriochloroform): δ 168.8, 148.8, 139.0, 138.0, 136.3, 133.8, 132.8, 132.5, 130.6, 128.9, 128.1, 127.3, 126.4, 122.3, 121.8, 116.8, 81.6, 66.5, 15.4. The ^{1}H NMR spectrum for **Target Compound 6** is shown below in Figure 18.

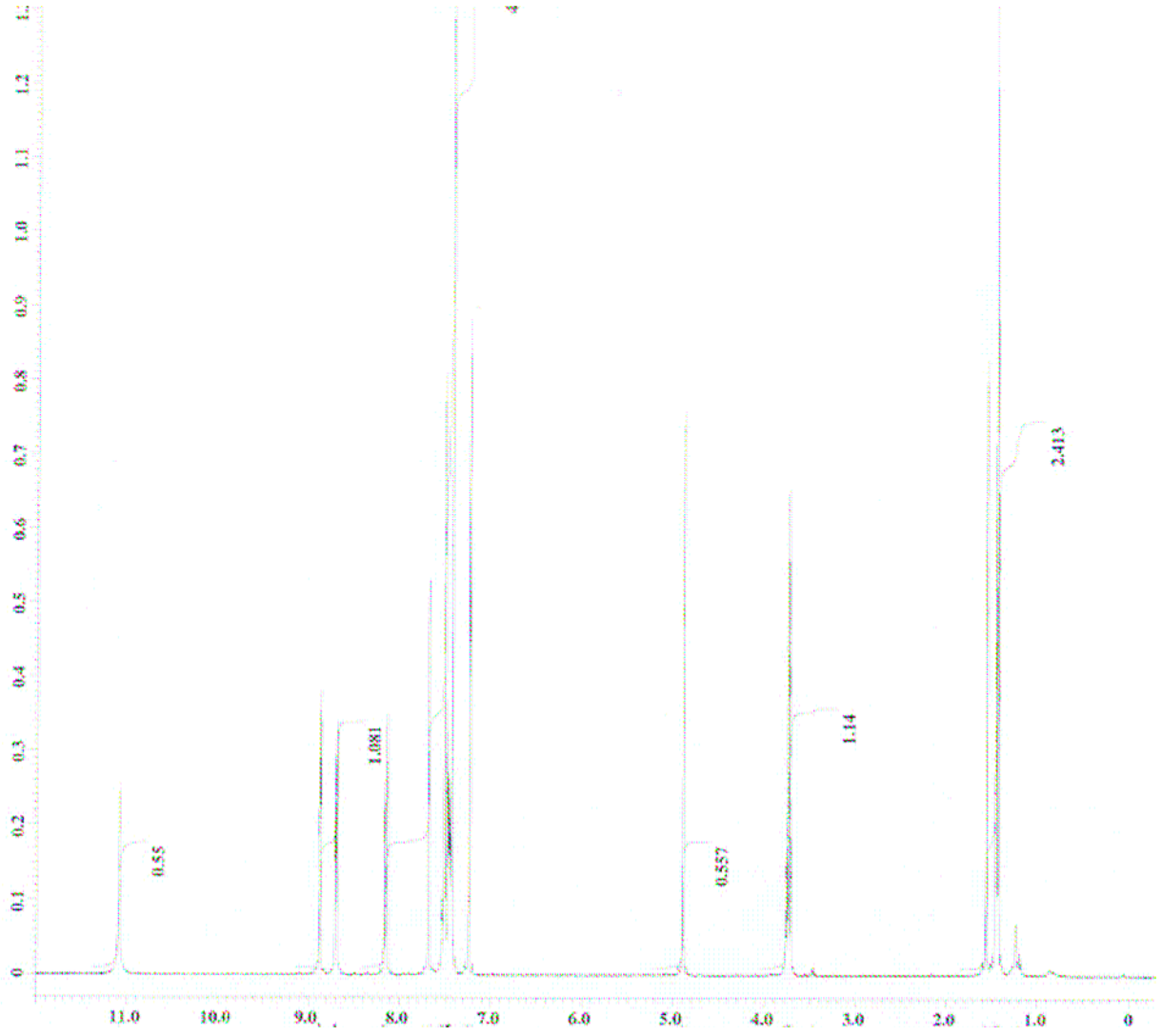

Figure 18: ^{1}H NMR spectrum of **Target Compound 6**